

WHAT IS CLAIMED IS:

- 1 1. An oligonucleotide primer comprising in the following order from 5'
2 to 3':
3 a phage-encoded RNA polymerase recognition sequence,
4 a spacer sequence comprising a sequence of from 12 to 20 nucleotides
5 that consists of one nucleotide type or two different nucleotide
6 types, and
7 a target complementary sequence which can bind a segment of a target
8 nucleic acid.

- 1 2. The primer of claim 1, wherein said spacer sequence comprises a
2 nucleotide sequence having the formula $(XY)_n$,
3 wherein n is from 6 to 10,
4 wherein X and Y are independently selected from the group consisting of an
5 adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a
6 thymidine nucleotide,
7 wherein X and Y are not the same.

- 1 3. The primer of claim 2, wherein X is an adenine nucleotide and Y is a
2 guanine nucleotide.

- 1 4. The primer of claim 3, wherein n is 9.

- 1 5. The primer of claim 1, wherein said spacer sequence comprises a
2 nucleotide sequence having the formula $(X)_n$,
3 wherein n is from 12 to 20,
4 wherein X is selected from the group consisting of an adenine nucleotide, a
5 guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide.

- 1 6. The primer of claim 5, wherein n is 18.

- 1 7. A method of amplifying a target nucleic acid in an aqueous solution
2 with a first and a second primer, said method comprising:
3 i.) transcribing an intermediate duplex with a phage-encoded RNA
4 polymerase to form a sense transcription product having a 5' end and a 3' end,

5 wherein said intermediate duplex comprises a double-stranded
6 molecule, wherein said double-stranded DNA molecule
7 comprises a first and a second strand,
8 wherein said first strand comprises in the following order from 5' to 3':
9 a phage-encoded RNA polymerase recognition sequence,
10 a first spacer sequence comprising a sequence of from 12 to 20
11 nucleotides that consists of one nucleotide type or two
12 different nucleotide types, and
13 a first target complementary sequence which can bind to a
14 segment of said target nucleic acid,
15 wherein said second strand comprises in the following order from 5' to
16 3':
17 a second target complementary sequence which can bind to a
18 segment of said target nucleic acid,
19 a second spacer sequence comprising a sequence of from 12 to
20 20 nucleotides that consists of one nucleotide type or
21 two different nucleotide types, and
22 a phage-encoded RNA polymerase recognition sequence,
23 wherein said transcribing takes place in the presence of Mn⁺⁺, with all
24 four dNTPs, and with those rNTPs represented in said first
25 spacer sequence;

- 26 ii.) hybridizing said second primer to said sense transcription product to
27 form a second primer-sense transcription product complex,
28 wherein said second primer comprises in the following order from 5'
29 to 3':
30 a phage-encoded RNA polymerase recognition sequence,
31 said second spacer sequence, and
32 said second target complementary sequence which can bind to
33 a 3' segment of said target nucleic acid;

- 34 iii.) extending said second primer-sense transcription product complex with
35 a Reverse Transcriptase that lacks RNaseH activity to form a first amplification duplex;
36 iv.) transcribing said first amplification duplex with a phage-encoded RNA
37 polymerase, in the presence of Mn⁺⁺, with all four dNTPs, and with those rNTPs represented
38 in said second spacer sequence, to form an antisense transcription product;

v.) hybridizing said first primer to said antisense transcription product to form a first primer-antisense transcription product complex,
wherein said first primer comprises in the following order from 5' to 3':

a phage-encoded RNA polymerase recognition sequence,
said first spacer sequence, and
said first target complementary sequence which can bind to a
5' segment of said target nucleic acid;

vi.) extending said second primer-antisense transcription product complex by Transcriptase that lacks RNaseH activity to form a second amplification

vii.) transcribing said second amplification duplex with a phage-encoded RNA polymerase, in the presence of Mn^{++} , with all four dNTPs, and with those rNTPs represented in said first spacer sequence to form said sense transcription product.

8. The method of claim 7, wherein the method further comprises repetitively carrying out steps i to vii.

9. The method of claim 7, wherein said first or said second spacer sequence comprises a nucleotide sequence having the formula $(XY)_n$, wherein n is from 6 to 10, wherein X and Y are independently selected from the group consisting of an adenine nucleotide, a guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide, wherein X and Y are not the same.

1 10. The method of claim 9, wherein X is an adenine nucleotide and Y is a
2 guanine nucleotide.

11. The method of claim 10, wherein n is 9.

12. The method of claim 10, wherein the rNTPs are rATP and rGTP.

1 13. The method of claim 7, wherein said first or said second spacer
2 sequence comprises a nucleotide sequence having the formula $(X)_n$,
3 wherein n is from 12 to 20,

4 **wherein X is selected from the group consisting of an adenine nucleotide, a**
5 **guanine nucleotide, a cytosine nucleotide, and a thymidine nucleotide.**

1 14. The method of claim 13, wherein n is 18.

1 15. The method of claim 7, wherein said sense and antisense transcription
2 products comprise a nucleic acid strand comprising both ribonucleotides and
3 deoxyribonucleotides.

1 16. The method of claim 7, wherein said first and said second
2 amplification duplexes consist of deoxyribonucleotides and ribonucleotides.

1 17. The method of claim 7, wherein said method is carried out at a single
2 temperature.

1 18. The method of claim 7, wherein said method is carried out at a single
2 temperature of between 25 °C and 55 °C.

1 19. The method of claim 1, wherein the method is carried out at a single
2 temperature of greater than 50 °C.

1 20. The method of claim 7, wherein said intermediate duplex comprises a
2 double-stranded DNA comprising one complete primer sequence followed by the entire
3 sequence that is to amplified.

1 21. The method of claim 7, wherein said intermediate duplex is formed
2 from double-stranded DNA, single-stranded DNA, or RNA.

1 22. The method of claim 7, wherein said intermediate duplex is formed by
2 the process comprising the following steps of:

3 denaturing a double-stranded DNA target to form an upper strand and a lower
4 strand;

5 hybridizing said first primer to said lower strand to form a first primer-lower
6 strand complex;

7 extending said first primer-lower strand complex with a Reverse Transcriptase
8 that lacks RNaseH activity or with a DNA Polymerase to form a first long sense strand
9 product-lower strand complex;

10 denaturing said first long sense strand product-lower strand complex into a
11 first long sense strand product and said lower strand;
12 hybridizing said second primer to said first long sense strand product to form a
13 second primer-first long sense strand product; and
14 extending said first primer-first long antisense strand product with a Reverse
15 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said
16 intermediate duplex.

1 23. The method of claim 7, wherein said intermediate duplex is formed by
2 the process comprising the following steps of:

3 denaturing a double-stranded DNA target to form an upper strand and a lower
4 strand;

5 hybridizing said first primer to said lower strand to form a first primer-lower
6 strand complex;

7 extending said first primer-lower strand complex with a Reverse Transcriptase
8 that lacks RNaseH activity or with a DNA Polymerase to form a first long sense strand
9 product-lower strand complex, wherein said first long sense strand product has a 5' and a 3'
10 end;

11 displacing said first sense strand product from said lower strand by:

12 hybridizing a bumper oligonucleotide to a subsequence on said lower
13 strand adjacent to said 5' end of said first sense strand product
14 on the first sense strand product-lower strand complex;

15 extending said bumper oligonucleotide with a Reverse Transcriptase
16 that lacks RNaseH activity or with a DNA Polymerase,

17 thereby displacing said first sense strand product;

18 hybridizing said second primer to said first long sense strand product to form a
19 second primer-first long sense strand product; and

20 extending said first primer-first long antisense strand product with a Reverse
21 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said
22 intermediate duplex.

1 24. The method of claim 7, wherein said intermediate duplex is formed by
2 the process comprising the following steps of:

3 hybridizing said second primer to a target RNA molecule to form a second
4 primer-RNA template complex;
5 extending said second primer-target RNA molecule complex with a Reverse
6 Transcriptase that lacks RNaseH activity or a DNA Polymerase to form a first long antisense
7 strand product-template complex, wherein said first long antisense strand product has a 5'
8 and a 3' end;
9 displacing said first long antisense strand product from said target RNA
10 molecule by:
11 hybridizing a bumper oligonucleotide to a subsequence on said target
12 RNA molecule adjacent to said 5' end of said first sense strand
13 product on the first sense strand product-lower strand complex;
14 extending said bumper oligonucleotide with a Reverse Transcriptase
15 that lacks RNaseH activity or with a DNA Polymerase,
16 thereby displacing said first long antisense strand product;
17 hybridizing said first primer to said first long antisense strand product to form
18 a first primer-first long antisense strand product complex; and
19 extending said first primer-first long antisense strand product with a Reverse
20 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said
21 intermediate duplex.

1 25. The method of claim 7, wherein said intermediate duplex is formed by
2 the process comprising the following steps of:
3 hybridizing said second primer to a single-stranded target RNA molecule to
4 form a second primer-RNA template complex;
5 extending said second primer-RNA template complex with a Reverse
6 Transcriptase that lacks RNaseH activity or a DNA Polymerase to form a first long antisense
7 strand product-template complex;
8 denaturing said first long antisense strand product-RNA template complex into
9 a first long antisense strand product and said single-stranded RNA molecule;
10 hybridizing said first primer to said first long antisense strand product to form
11 a first primer-first long antisense strand product complex; and
12 extending said first primer-first long antisense strand product with a Reverse
13 Transcriptase that lacks RNaseH activity or with a DNA Polymerase to yield said
14 intermediate duplex.

1 26. The method of claim 7, wherein said phage-encoded RNA
2 polymerase is polymerase selected from the group consisting of : a T7 RNA polymerase,
3 a T4 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase and a K11 RNA
4 polymerase.

1 27. The method of claim 26, wherein said phage-encoded RNA
2 polymerase is a mutant phage-encoded RNA polymerase that is competent to incorporate
3 dNTPs into a template nucleic acid.

1 28. The method of claim 27, wherein said phage-encoded RNA
2 polymerase is a T7 RNA polymerase.

1 29. The method of claim 28, wherein said T7 RNA polymerase
2 contains a Y639F mutation.

1 30. The method of claim 28, wherein said T7 RNA polymerase
2 contains a S641A mutation.

1 31. The method of claim 28, wherein said T7 RNA polymerase
2 contains at least two mutations.

1 32. The method of claim 7, wherein said Mn⁺⁺ is present in a
2 concentration of between 10 µM to 20 mM.

1 33. The method of claim 32, wherein said concentration is 10 mM.

1 34. The method of claim 7, wherein said target nucleic acid is single-
2 stranded DNA.

1 35. The method of claim 7, wherein the target nucleic acid is
2 comprised of RNA.

1 36. The method of claim 7, further detecting said sense transcription
2 product, said antisense transcription product, said first amplification duplex, or said
3 second amplification duplex,

4 wherein said detecting comprises hybridizing a detection oligonucleotide
5 comprising a detectable moiety, wherein said detection oligonucleotide is complementary

6 to a subsequence of said sense transcription product, said antisense transcription product,
7 said first amplification duplex, or said second amplification duplex.

1 37. A kit for copying a target nucleic acid comprising:
2 a container containing:
3 a first nucleotide primer comprising in the following order from 5' to 3':
4 a phage-encoded RNA polymerase recognition sequence,
5 a first spacer sequence comprising a sequence of from 12 to 20
6 nucleotides that consists of one nucleotide type or two
7 different nucleotide types,
8 a first target complementary sequence which can bind to a
9 segment of said target nucleic acid; and
10 a second primer comprising in the following order from 5' to 3':
11 a phage-encoded RNA polymerase recognition sequence,
12 a second spacer sequence comprising a sequence of from 12
13 to 20 nucleotides that consists of one nucleotide
14 type or two different nucleotide types, and
15 a second target complementary sequence which can bind to
16 a segment of said target nucleic acid.

1 38. The kit of claim 37, wherein said phage-encoded RNA polymerase
2 is polymerase selected from the group consisting of : a T7 RNA polymerase, a T4 RNA
3 polymerase, a T3 RNA polymerase, a SP6 RNA polymerase and a K11 RNA polymerase.

1 39. The kit of claim 38, wherein said phage-encoded RNA polymerase
2 is a mutant phage-encoded RNA polymerase that is competent to incorporate dNTPs into
3 a template nucleic acid.

1 40. The kit of claim 38, wherein said phage-encoded RNA polymerase
2 is a T7 RNA polymerase.

1 41. The kit of claim 40, wherein said T7 RNA polymerase contains a
2 Y639F mutation.

1 42. The kit of claim 40, wherein said T7 RNA polymerase contains a
2 S641A mutation.

1 43. The kit of claim 40, wherein said T7 RNA polymerase contains at least
2 two mutations.

1 44. The kit of claim 37, further comprising a member selected from the
2 group consisting of:
3 a DNA polymerase;
4 a Reverse Transcriptase that lacks RNaseH activity;
5 a phage-encoded RNA polymerase;
6 all four dNTPs;
7 those rNTPs represented in said first and second spacer sequences;
8 reaction buffer containing manganese in a concentration from 10 µM to 20
9 mM;
10 a positive control target nucleic acid; and
11 instructions for carrying out a method of copying a nucleic acid using said first
12 primer and said second primer.